Syllabus

HEMATOLOGY

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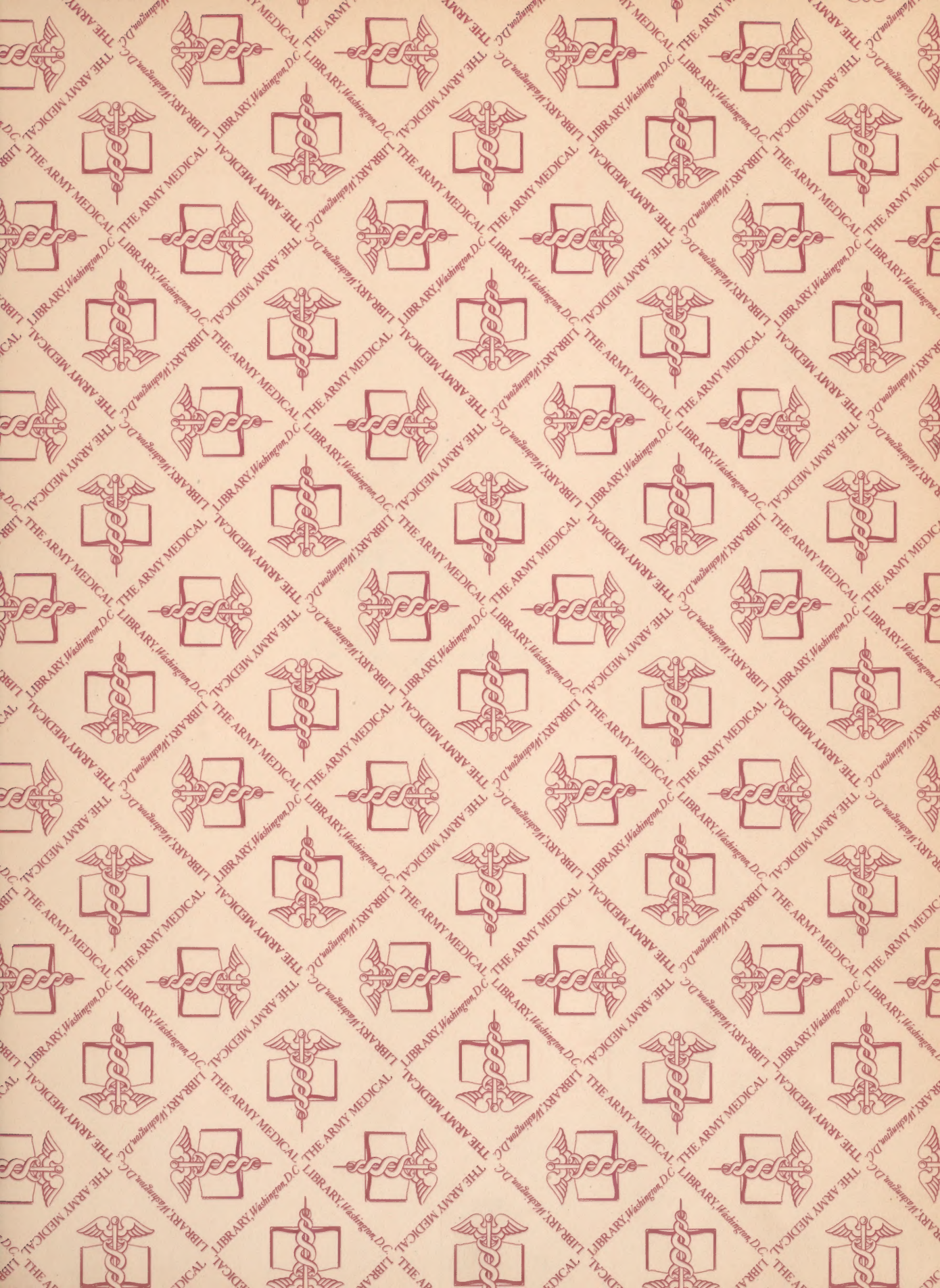
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COMPOSITION OF THE BLOOD

The blood is composed of a liquid portion, the plasma, and of the formed elements or cells suspended in the plasma. The plasma consists of water, dissolved salts, food, waste products and proteins, including the protein fibrinogen which plays an important part in blood clotting. The formed elements consist of the red and white blood cells and of blood platelets. All of these constituents are subject to considerable normal variations, in accordance with age, sex, and living conditions.

The red blood cells, or erythrocytes, are small biconcave discs, averaging 7.7 Mu in diameter. Their number varies between 4.5 and 5.5 millions per cubic millimeter of blood in most normal individuals. These cells have a thin wall enclosing a jelly-like mass containing the iron-protein compound called hemoglobin, wiich gives the characteristic red color to the blood. Hemoglobin combines with oxygen in the lungs and transports it throughout the body. The amount of hemoglobin in the blood varies with its concentration in the blood cells and with the number of red blood cells present. The average normal person has from 14 to 17 gms. of hemoglobin per 100 cubic centimeters of blood.

The white blood cells, or leukocytes, are fewer in number than the erythrocytes, averaging from 5000 to 10,000 per cubic millimeter of blood. They are larger in size, measuring 10-15 mu in diameter and are divided into several groups depending on their nuclear structure and on the granules found within their cytoplasm. Roughly, they are divided into cells with a single round or indented nucleus (mononuclear cells) and into cells with lobulated nuclei which are known as polymorphonuclear cells, or granulocytes, because their cytoplasm contains numerous granules. A more detailed description of these cells is provided in the section on differential blood counts.

The platelets, or thrombocytes, are small refractile bodies 2 - 3 mu in diameter. There are 200,000 to 400,000 thrombocytes per cubic millimeter of blood. When a blood vessel is cut and blood is shed, the platelets disintegrate, producing thromboplastin. Through a complex process, involving a calcium of the blood, the fibrinogen is converted into fibrin which is the thread-like material forming a blood clot. Normal blood clots in 2 to 8 minutes.

In sick people, these blood constituents may show marked changes which are helpful in establishing the diagnosis and following the progress of the disease. It is the object of hematology to measure these changes as accurately as possible to achieve this purpose.

EXAMINATION OF THE BLOOD

The routine laboratory work in hematology requires very small amounts of blood, which may be obtained from the finger or ear in adults or from the bottom of the heel in infants. Usually the ball of the middle or ring finger is used. Some of the more technicil tests require larger amounts of blood and it is necessary to puncture a vein.

FINGER PUNCTURE

Materials

1. A sharp cutting edge is necessary, never use a round needle or pin, because the hole closes too quickly.

   a. Use a Hagedorn needle (cutting edge) or,

   b. Automatic blood lancet, or,

   c. Bard-Parker blade (size eleven). This blade may be pushed through a cork and this cork used to stopper the alcohol

bottle. Keep all the cutting blades as clean and shiny as possible.

2. Cotton
3. 70% Alcohol
4. Clean pipettes and chemically clean slides.

Procedure:

1. Rub the patient's finger briskly or place the hand in warm water to promote blood flow.
2. Clean the finger with the alcohol and dry. If the finger tip is wet the blood will not form a round drop.
3. Hold the ball of the finger tightly between the operator's thumb and index finger, until the skin color is dark red. Puncture the finger with a firm, quick stroke, deep enough so the blood will flow immediately. Do not squeeze the finger after the puncture because this forces tissue juices into the cut and dilutes the blood.
4. Wipe off the first drop with dry cotton.
5. Allow a sufficiently large drop form, before touching a blood pipette or slide to the drop. Fill the pipettes and make smears as indicated.

BLEEDING TIME

Bleeding time is the time that it takes the blood to stop flowing from a measured cut in the finger or ear.

Materials:

1. Finger puncture equipment
2. Filter paper
3. Watch.

Procedure:

1. Puncture the finger or ear lobe.
2. Note time when blood begins to flow.
3. Blot with the filter paper every ½ minute.

4. The time between the first drop and the last is the bleeding time. Normal bleeding time is from 1 to 3 minutes. When the bleeding continues longer than 10 minutes the bleeding time is seriously prolonged.

COAGULATION TIME

This represents the length of time it takes a specimen of blood to clot so that fibrinogen is converted into strands of fibrin. Normal coagulation time is from 2 - 8 minutes.

I. Slide Method:

Material:

1. Finger puncture equipment
2. Clean Slide.
3. Needle
4. Watch

Procedure:

1. Clean the finger with alcohol and make a routine puncture.
2. Place a few drops of blood on the slide.
3. At $\frac{1}{2}$ minute intervals draw a needle slowly through the blood drop. When a fine thread (fibrin) can be picked up by the needle point, coagulation has begun.
4. The time between the flow of blood and the formation of fibrin is coagulation time.

II. Capillary Tube Method:

Draw out soft glass tubing into a capillary pipette, over a wing top bunsen burner. After finger puncture fill the tube, then at $\frac{1}{2}$ minute intervals break off 3-4 mm. of the tube. If the fibrin strings out at the broken end coagulation has begun. Normal coagulation time is from 2 to 8 minutes.

DETERMINATION OF HEMOGLOBIN

Hemoglobin is measured in grams per hundred cubic centimeters of blood or as a percentage of an arbitrary normal standard. These figures may be obtained by comparing the color of the blood to be examined with the color of blood whose hemoglobin concentration is known. This principle is utilized in the Tallquist method by placing a drop of the patient's blood on a piece of special absorbent paper and comparing it with color plate reproductions of blood containing various amounts of hemoglobin. The color plate which most nearly matches the patient's blood represents the percentage of hemoglobin. In the Tallquist scale a concentration of 15.8 gm. of hemoglobin per 100 cc of blood represents 100%. The color plates are arranged in descending gradations of 10%, but values higher than 15.8 gm. can not be determined. Due to the difficulty in matching colors this method requires daylight and is at best quite inaccurate so that it is only used for rough estimations.

A more accurate method involves the dissolution of a measured amount of blood with 1% hydrochloric acid. The red cells are laked (dissolved) and the hemoglobin is converted into a reddish brown compound, acid hematin. The color produced is proportional to the amount of hemoglobin present and this may be determined by comparison with either a known solution of acid hematin, or with a standard colored glass; or the intensity of color may be measured by means of a photo-electric cell. Instruments for such measurements are called hemometers or hemoglobinometers.

A. Tallquist Method:

Materials:

1. Finger puncture equipment.
2. A Tallquist scale which is a sheet of paper with spots of red color on it, graded to represent hemoglobin content from 10% to 100%.

3. Absorbent paper supplied in a book with the scale.

Procedure:

1. Blot a drop of blood with a thin slip of the absorbent paper. Set aside to dry. Make the reading as soon as dry.
2. Match against the color standard using a white background, in daylight if possible. This test is the most commonly used, but is very inaccurate and it is only possible to show gross changes. The scale is based on 15.8 grams of hemoglobin per 100 cc. of blood equals 100%. Reports are expressed in percentages 90-80-70- etc.

B. The Photoelectric Hemoglobinometer (Fischer)

Materials:

1. Finger puncture equipment.
2. 5 cc hemoglobin pipette.
3. 1% hydrochloric acid.
4. Photoelectric hemometer.

Procedure:

1. Secure a large drop of blood from the finger or ear.
2. Draw the blood exactly up to the 20 c.m.m. mark; wipe off the excess of blood from the tip; and fill the pipette up to the 251 mark above the bulb. This gives a dilution of 1:251.
3. Mix the blood well with the acid by shaking and let stand for 25 minutes.
4. Operation of the Photoelectric cell:

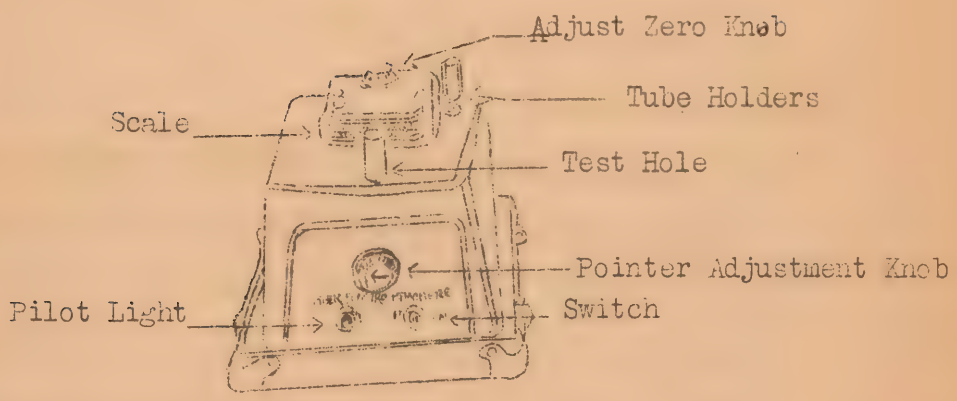
   (a) Construction:

   The photoelectric hemometer consists of:

   (1) a galvanometer needle and scale.

   (2) a photoelectric cell connected to the galvanometer.

FISHER ELECTRO-HEMOMETER



(3) a constant light shining on the photoelectric cell.

(4) a tube holder between the light and the photoelectric cell.

(5) two standard test tubes, one sealed containing distilled water, the other empty for the specimen of blood to be examined.

(6) a control knob (marked "Adjust Zero") for setting the galvanometer needle.

(7) a rheostat control for the light located on the front panel. ("Pointer Adjustment Knob")

(b) Taking the reading

(1) the galvonometer needle is extremely sensitive and is easily displaced and damaged by jarring. For this reason once the hemometer is set up for operation it should not be removed. If the galvanometer needle has been jarred from the starting mark (A) it is brought back to the place by slowly twisting the "adjust zero" knob before turning on the light:

(2) The current is then turned on and the instrument allowed to "warm up" for 2 minutes because the photoelectric cell does not reach its maximum efficiency until that time has elapsed.

(3) The tube containing distilled water is now placed in the holder between the light and the photoelectric cell. By means of the rheostat knob the galvanometer needle is brought to the line B. The instrument is now ready for operation. This adjustment must be made every time a reading is made.

(4) The acid hematin solution in the pipette is expelled into the specimen tube (allowing for full development of color) and the specimen tube is placed in the reading rack. The galvanometer needle will now swing from line B toward line A and after several oscillations will come to rest on the scale representing the hemoglobin concentration. The reading is recorded and the specimen tube is replaced by the distilled water tube which should bring the needle back to line B. The reading is repeated.

(5) If the reading does not check within .2 gm. or the needle does not return to line B, the hemoglobinometer should be readjusted and new readings taken.

(6) NOTE: The direction given above are for the Fischer photohemometer. Instruments made by other manufacturers work on a similar principle but have special operating details. Be sure to read the instructions before using these instruments. Also, all dirt and finger marks must be wiped off the tubes before using.

(c) Reports:

The Fischer hemometer should be read and reported directly in grams. The percentage scale corresponds to 15.6 gm. as 100% and percentages should be converted to correspond to the standard of this laboratory in which 16.6 Gm. is 100%.

C. The Newcomer Hemoglobinometer.

Materials:

1. Finger Puncture equipment.

2. 5 c.c. hemoglobin pipette.

3. 1% hydrochloric acid.

4. Newcomer hemoglobinometer.

Procedure:

1. Secure a drop of blood from the finger or ear.

2. Draw the blood exactly up to the 10 c.m.m. mark; wipe off the excess of blood from the tip and fill the pipette up to the 251 mark above the bulb. This gives a dilution of 1:501.

3. Mix the blood well with the acid by shaking and let stand for 20 minutes.

4. Operation of the hemoglobinometer.

   (a) Construction and operation:

   The apparatus consists of a colorimeter arrangement in which the standard cup is filled with distilled water and a color disc is interposed between this cup and the eyepiece. The unknown cup is filled by the acid hematin solution and the colors matched by moving the cup up or down. The concentration of hemoglobin in grams may then be directly read off the scale on the adjustment knob.

5. The readings are made directly in grams and then expressed in percent of the laboratory standard (16.6 grams.)

Note: When 5 cc. hemoglobin pipettes are not available the blood may be Drawn with the 20 c.m.m. pipette and expelled into a test tube containing exactly 10 c.c. of 1% hydrochloric acid.

Red Blood Cell Counting:

A. Materials:

1. Lancet for puncture.

2. Cotton

3. Water, alcohol, and ether for cleaning pipettes.

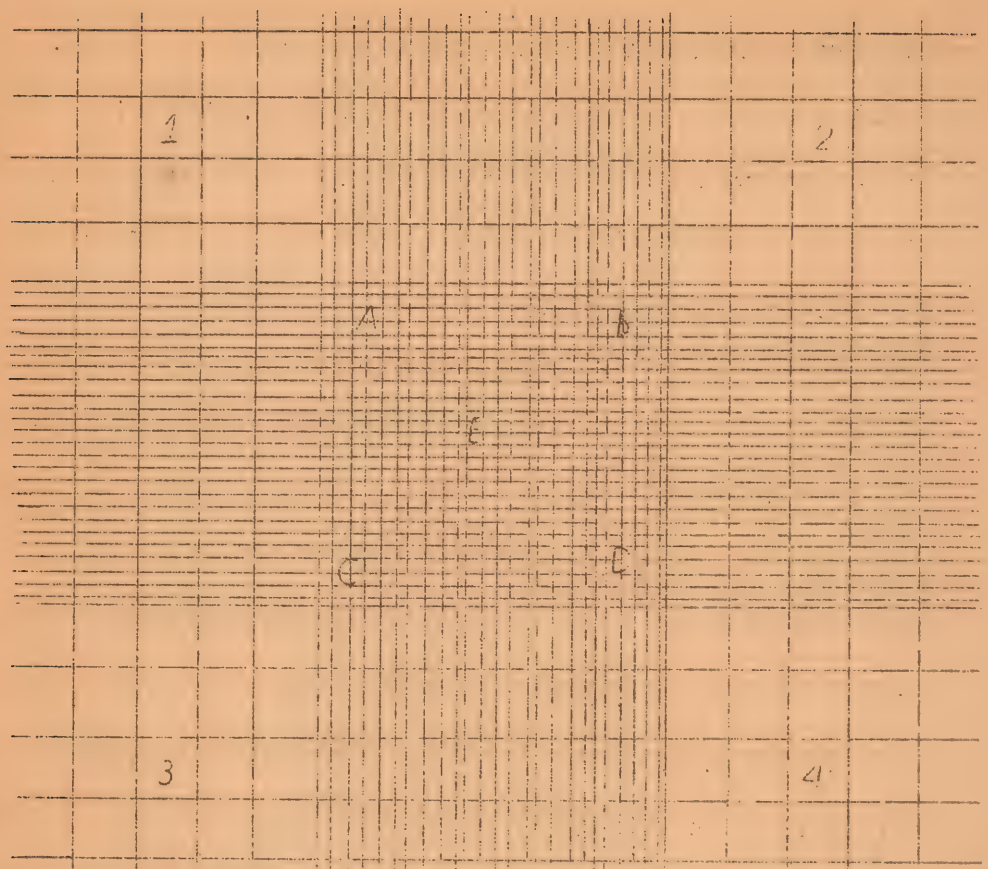
4. Microscope.

5. Diluting pipette for red blood cells. It often has a red bead in the bulb to make it quickly recognizable. The Thoma pipette is marked in graduated lines along the capillary bore. The fifth graduation from the tip is marked 0.5, the tenth, 1.0; above the bulb is a line marked 101. In this pipette, if blood is drawn to the 0.5 mark and the diluting fluid to the 101 mark, the dilution is 1 to 200.

6. Counting chamber. The Levy chamber with the improved Neubauer ruling is the supply table item of issue. There are other types of ruling and several kinds of chambers, all similarly used. The chamber is a thick glass slide with two central platforms; on the surface of each is engraved a series of rulings. The side platforms on which the special cover glass fits are exactly 0.1 mm higher than the central platforms. When the cover slip is in place there is a space 0.1 mm deep, the ruled areas having a surface area of 9 sq. mm. The four large corner squares outside the double ruled lines (marked 1,2,3,and 4) are each subdivided into 16 smaller squares. The central square is divided by double lines into 25 small squares each of which contains 16 smaller squares, making a total of 400 squares (see fig. 1) Each small square then is 1/400 sq. mm.

Note: The method of blood counting is thoroughly explained herein. The number 1,2,3, and 4, and the 16 surrounding squares of each, indicate the parts of the slide used in counting white blood cells. The letter A,B,C,D, and E, and the areas between the double lines, indicate the areas used in counting red blood cells.

Figure 1.--Improved Neubauer counting chamber.



Note: The method of blood counting is thoroughly explained herein. The nos.1,2, 3 and 4, and the 16 surrounding squares of each, indicate the parts of the slide used in counting white blood cells. The letters A,B,C,D, and E, and the areas between the double lines, indicate the areas used in counting red blood cells.

7. Diluting fluid- Hayems solution:

Sodium chloride	1.0 gm.
Sodium sulfate	5.0 gm.
Mercuric chloride	0.5 gm.
Distilled water	200 cc.

B. Counting Chamber and pipette cleaning:

All pipettes and counting chambers should be clean and dry before using and should be cleaned immediately after using. Avoid harsh rubbing or strong solutions on the counting chamber.

1. Counting chamber: Cleanse the surface of the counting chamber with soap and water. Wash with distilled water and air-dry. If needed immediately, dry on soft gauze and lens paper. Cleansing with xylol and other cement solvents must be avoided; alcohol or ether may be used, with care to remove oil.

2. Pipette.

   (a) Draw water through pipettes by suction (mouth suction, water pump, or air pump).

   (b) Draw alcohol through pipettes by suction; this will remove the water.

   (c) Draw ether through pipettes; this will remove the alcohol. Continue the suction of air for a few seconds to remove the ether.

   (d) The small bead in the bulb should then shake about freely, indicating a clean and dry pipette. If pipettes become plugged through neglect, clean capillary bore with a horse-hair and soak overnight in dilute nitric acid, then clean as above.

C. Procedure:

1. Puncture the finger in the usual way.

2. Draw up blood exactly to the 0.5 mark on the red blood cell pipette. Remove any excess on the outside of the tip by wiping on a piece of gauze.

3. Draw up diluting fluid exactly to the mark 101, making a dilution of 1 to 200.

4. Kink the rubber tube at the end of the pipette, hold it against the middle finger with the capillary point on the ball of the thumb, and shake in a figure-of-eight motion for 2 minutes to

insure good mixing.

5. Put the cover slip in place on the counting chamber.
6. Blow out 3 drops, touch the tip of the pipette to the edge of the platform, and allow a thin layer of fluid to flow under the cover glass. If the fluid flows into the troughs, or there are bubbles under the cover glass, clean the counting chamber and try again.
7. Allow the cells to settle for 2 minutes.
8. Examine under the high-dry lens of a microscope.

D. Counting:

Count all the cells in squares A,B,C,D, and E, as illustrated in figure 1. In counting cells in each square (as A in fig. 1) enclosed by double lines, count all cells touching the inner lines on the right and top of the square. Do not count any cells touching the lines on the left and bottom of the square. The difference between the number of cells in any two blocks should not be more than 15 cells. If this is the case, the mixing was not complete or the chamber was dirty. From this count calculation is made of the number of cells per cubic millimeter of blood.

E. Calculation Example:

1. Long method:

(a) Squares A,B,C,D, and E, give counts 100, 98, 104 and 100; total, 500.

(b) Therefore, 80 small squares, which occupy 5/25 or 1/5 sq. mm., contain 500 cells.

(c) One square millimeter would contain 5 x 500 = 2,500 cells.

(d) As this cell layer is 0.1 mm thick, 1 cu mm would contain 10 x 2,500 = 25,000.

(e) As the blood was diluted 200 times: 200 x 25,000 = 5,000,000

cells per cubic millimeter of blood.

(f) Summary: Count of 80 small squares x 5 (for area) x 10 (for volume) x 200 (for dilution) equals number of cells per cubic millimeter of blood.

2. Short method:

If the dilution was 1:200, the total cells per cubic millimeter may be found by adding four zeros to the total red blood cell count in squares A,B,C,D, and E. For example, 500 with four zeros added will be 5,000,000.

F. Normal red blood cell (erythrocyte) counts:

Men: 4,500,000 to 6,000,000 per cu mm.

Women: 4,000,000 to 5,500,000 per cu mm.

G. Sources of error

(1) Failure to hit the mark exactly with blood.

(2) Inaccurate dilution--either a bubble in pipette or failure to exactly hit the mark with diluting fluid.

(3) Improper placing of the coverslip on the chamber.

(4) Overfilling the chamber.

(5) Not shaking long enough in the pipette.

(6) Dirty chamber or pipette.

(7) Yeast growing in the diluting fluid.

COLOR INDEX:

A. The term "color index" means the amount of hemoglobin in the average red cell of the patient compared with the normal amount.

$$\text{Color index} = \frac{\text{hemoglobin percent}}{\text{red blood cells percent}}$$

B. To express the red blood cell count as percent, it is necessary only to multiply the first two figures of the total red blood cell count by 2.

Example:

Red blood cells, 5,000,000.

Hemoglobin, 100 percent.

Color index $\frac{100}{50 \times 2} = 1$.

c. A normal color index ranges from 0.85 to 1.15.

26. White blood cell (leucocyte) counting.

a. Materials

(1) Same as for red blood cell count except for the pipette and the diluting fluid.

(2) White blood cell pipette is similar to the red cell pipette, but has a smaller bulb which contains a small white bead and gives less dilution to the blood. The fifth line on the graduated capillary tube is marked 0.5, the tenth line 1.0, and the above the bulb 11.

(3) White blood cell diluting fluid:

Glacial acetic acid - - - - - - - - - - - - - - - 0.5 cc

Distilled Water - - - - - - - - - - - - - - - - -99.5 cc

This fluid may be tinted blue, for convenience in identifying it, by addition of a drop of 1 percent gentian voilet. This solution should be freshly prepared every two weeks.

b. Procedure.

(1) Draw blood to the 0.5 mark.

(2) Draw diluting fluid to the mark 11, making a dilution of 1:20.

(3) Shake as in red blood cell counting.

(4) Discard 3 or 4 drops and fill the counting chamber.

(5) Allow the cells to settle.

(6) Examine under the low power of the microscope.

(7) When doing a complete blood count, shake the red blood pipette in one hand, the white in the other. Fill the counting chambers, red on one side and white on the other.

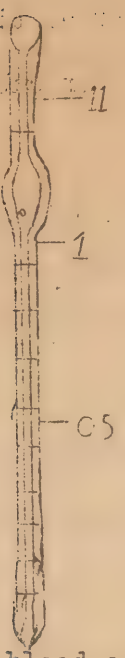


Figure 3.--White blood cell pipette.

c. Calculation.

The white cells are counted in the four large corner squares labeled 1, 2,3,4 in figure 1. The difference between the largest and smallest number of cells in any two squares should not exceed 10. Each large square contains 16 smaller squares and represents a volume of 0.1 cu mm. The 4 squares are counted and divided by 4 to get the average per 0.1 cu mm.

d. Example of calculation for white blood cells.

(1) Long method.

Square 1 ---------- 34

Square 2 ---------- 42

Square 3 ---------- 38

Square 4 ---------- 36

Total for 0.4 cu mm diluted blood (4 squares 0.1 mm thick) ---------- 150

Total for 0.1 cu mm diluted blood (150 ÷ 4) ---------- 37.5

Number of cells in 1 cu mm diluted blood (37.5 x 10)--- 375

To obtain the number in 1 cu mm of undiluted blood multiply by 20 (as the dilution is 1 to 20) or (20x375 = 7,500. Summary: Count of 4 squares,

divided by 4, times 10 (for area) times 10 (for volume) times 20 (for dilution) equals number of cells per cubic millimeter of blood.

(2) Short method:

Multiply the total number of cells in 0.4 cu mm by 50; 150 x 50 = 7,500 white cells per cu mm.

e. Normal white blood cell (leucocyte) count.

Normally, this count is 5,000 to 10,000 per cu mm.

Many normal persons have variable counts due to activity, time of day, etc. Daily counts on a patient should be done at the same time every day. In certain cases where the white count is very high, it may be necessary to use a dilution of 1:100, using the red cell pipette, changing the calculation accordingly. In cases where the count is abnormally low, make the dilution 1:10 by drawing blood to the 1.0 mark instead of 0.5.

27. Glassware cleaning (for blood films).

A prerequisite in making a good blood film is to have chemically clean glassware.

a. New slides,

(1) Wash in soapy water and rinse thoroughly with water.

(2) Place slides in a large beaker of 95 percent alcohol.

(3) Polish with a soft lint-free cloth (not gauze).

(4) Flame over a bunsen burner.

(5) Place in box with clean slip of paper between each slide.

b. Dirty slides.

(1) Boil in 5 percent sodium bicarbonate solution.

(2) Scrub with soap and water.

(3) Place in cleaning solution (potassium bichromate-sulfuric acid) for 12 hours.

(4) Then wash as for new slides.

(5) Discard all slides that are badly scratched or discolored.

The same as for slides except do not flame. Careful wiping will prevent much breakage. Do not use pressure.

28. Preparation of blood films.

a. Materials.

(1) Equipment for finger puncture.

(2) Clean slides free from grease.

b. Procedure.

(1) Puncture the finger.

(2) Place 1 small drop of blood on the end of a slide and place the slide on a flat surface.

(3) Hold a second slide between the thumb and third finger and place one end at a 30° angle on the slide holding the drop of blood.

(4) Pull the upper slide until it touches the drop of blood which then spreads along the narrow end of the top slide (see fig 4). (The slide held with the left hand is supposed to be laid flat on a smooth surface).

(5) Push the top slide with a firm, steady motion toward the opposite end of the bottom slide; the slower the movement, the thicker the film; the greater the angle, the thicker the film. Avoid all unnecessary pressure because of the fragility of the cells.

(6) Allow to air-dry. In areas where insects are abundant slides must be protected or they will be ruined. A good film should be smooth and without waves. The edges should be even and the film should not extend to the edges or end of the slide. Labelling may be done by writing on the thicker end of the film with an ordinary lead pencil when the film is dry. The slides should be stained within 24 hours for best results.

29. Blood stains. - Wright's stain.

This stain is used for routine blood films and for other laboratory purposes. The prepared

stains purchased by the Army are easy to prepare if specific directions are followed:

Materials:

1. Chemically clean glass ware;
2. Wright's stain prepared as follows:

   Wright's powder (supply table item)- - - - - - - - - - 0.3 Gm.

   Glycerine - 3.0 cc.

   Methyl alcohol absolute (must be acetone free- - q.s. 100.0cc

   Put the powder in a dry mortar grind with a pestle, add the glycerine and grind. Add the methyl alcohol and mix. Allow to stand overnight in a tightly stoppered flask, then filter and keep for a few days before use, age improves the stain.

3. Buffer solution:

   Potassium phosphate (monobasic) - - - - - - - - - - -1.13 Gm.

   Dibasic sodium phosphate - - - - - - - - - - - - - - 3.20 Gm.

   Distilled Water -1000.cc.

Procedure:

1. Fill a Coplin jar with stain and place the smears in the stain for 2 minutes. This fixes and stains the blood film.
2. Next, place the slides in another coplin jar filled with the buffer solution for 4 minutes.
3. Place the slides in a 3rd Coplin jar filled with distilled water and leave them until the blue color of the blood film commences to turn a pinkish color. As soon as this decolorization process is complete, remove the slides and dry them. They are now ready to be examined.

NOTE: The color of the cells may be varied by changing the stain or buffer time. The granules of the neutrophiles should stain a lilac

color, the eosinophiles a bright red, and the basophiles deep blue.

B. Giemsa Stain:

Materials:

1. Staining jars (Coplin jar)
2. Giemsa Stain
   (a) Stock solution

   Giemsa powder (supply table item) - - - -0.5

   Glycerine, dissolve powder in this 1 to 2 hours 33.0 cc.

   Methyl alcohol absolute (acetone free) 33.00 cc.

   (b) Dilute stain (ready for use)

   1 cc. stock solution to 10 cc distilled water.

3. Methyl alcohol.

A. Procedure:

(1) Fix smear with methyl alcohol 3 to 5 minutes in a Coplin jar.

(2) Dry in air.

(3) Put in dilute stain for 20 to 30 minutes (Coplin jar).

(4) Wash in distilled water.

(5) Stand on end to dry.

(6) Examine under oil immersion.

This stain is excellent for protozoal staining but is more time-consuming than the Wright's stain method.

DIFFERENTIAL WHITE BLOOD CELL COUNT

Materials:

1. Finger puncture and staining equipment.
2. Wright's stain.

Procedure:

1. Prepare films.
2. Stain with Wright's stain.

3. Examine under oil immersion, record each type of white cell seen.

Note: Survey the slide to determine the area that is neither too thick nor too thin, This place will usually be towards the latter portion of the slide. Start on one extreme side and cross the smear. Move one or two fields and again cross the slide being careful to always count the very edges. Count 200 cells.

CHARACTERISTICS OF STAINED CELLS

I. Red Blood Cells:

Normal red blood cells (erythrocytes) are round, non-granular, non-nucleated cells, the centers of which are less intensely and colored than the borders. In the various diseases the blood may contain erythrocytes showing the following abnormalities:

Achromia - Pale staining erythrocytes; decreased hemoglobin.

Polychromasia - Many of the erythrocytes take a bluish rather than a tan color.

Anisocytosis - A wide variation in the size of the cells.

Poikilocytosis - A wide variation in the shape of the cells.

Macrocytosis - The Average size of the cells is greater than the normal 7.5 mu.

Microcytosis - The average size of the cells is smaller than normal.

Stippling - The cells contain a fine dusting of bluish black granules, as seen in lead poisoning.

Howell-Jolly Bodies - The cells contain one or two small blue-black dots.

Reticulocytes - These red cells have a feathery, dark blue, irregular network within the cell when stained with Brilliant Cresyl Blue Stain.

II. White Blood Cells:

Lymphocytes are always mononuclear. The magnitude of the nucleus in

proportion to the size of the cell is their distinguishing feature. The amount of cytoplasm is relatively small forming a narrow ring around the nucleus. It contains no granules that are of any great diagnostic value. Lymphocytes may be divided into two divisions according to their size. The small ones constitute 20 to 25 percent of all leukocytes and are similar to the red cells in size. The large ones usually constitute 5 to 8 percent of all leukocytes, and are about twice the size of the erythrocytes. The small variety is the mature cell while the larger one is immature. Because of their usual lack of granules, lymphocytes are sometimes called agranulocytes.

The necleus of each type is usually round, but occasionaly flattened a little on one side. It stains a very deep blue while the cytoplasm is a very pale blue.

Monocytes: These are the largest cells found in the normal blood. The nucleus is often slightly irregular with a larger zone of cytoplasm than the Lymphocyte. The cytoplasm of the Monocyte is usually filled with fine azure dust-like gramules that give it a hazy appearance.

Polymorphonuclears are smaller than the monocytes, being about the size of the large lymphocytes. They are characterized by the various shapes of their nuclei. Each cell appears to have from two to five nuclei but on close inspection they are found to be connected.

Polymorphonuclear leukocytes are divided into three groups according to the reaction of their granules to the stain. Cells whose granules are small, taking a very light lilac stain, are called neutrophils and constitute about 65% of all leukocytes. When the granules take a very pronounced red or eosin stain, they are called eosinophils and are about 2% of all leukocytes. Those whose granules take a purple or basic stain are called basophils, and constitute about 0.5% of all leukocytes.

The nuclei of the neutrophils and eosinophils are very nearly the same shape, but in the basophils the nucleus is almost round.

Because of the peculiar reaction of the granules to the stain, this group is sometimes called granulocytes.

The abnormal forms of leukocytes are myeloblasts, myelocytes, juveniles, lymphoblasts, monoblasts and a few atypical forms.

The myeloblasts are the grand-parents of the polymorphonuclears. They range between 15 and 16 microns in diameter. The cytoplasm forms a very narrow rim which stains blue with a diffused effect, being free from granules. The nucleus is round and sharply defined. It contains several nucleoli. Occasionally, vacuoles are present in the cell. These cells are never found in a healthy blood stream.

The abnormal form of leukocytes most commonly seen is the myelocyte. It is larger than any cell found in the normal blood. The nucleus occupies about one-half the entire cell. It is usually flat or concave on one side lying close to the cell wall. The granules vary in size and react differently to the stain. They can be classed as neutrophilic, eosinophilic or basophilic. The myelocytes are direct descendants of the myeloblasts and about the same size but differ since they have granules.

Juveniles are a group of cells emphasized by Schilling. They represent a transitional stage between the Myelocyte and the Polymorphonuclear neutrophil. They are smaller than the Myelocyte and the nucleus is slightly indented, like a kidney bean. The cytoplasm of the Juvenile is neutrophilic, Eosinophilic, or Basophilic.

Stabs are just a little more mature than are the Juveniles. The nucleus of the Stab cell looks like a straight or curved rod and may be slightly constricted or bulged in different places, but never segmented. As Juveniles, so also the cytoplasm of Stabs may be Neutrophilic, or Basophilic. Normally about 4% of all Leukocytes are Stabs.

The Lymphoblasts are the parent cells of lymphocytes and are never found in normal blood. They are larger than the lymphocytes, but in other respects, very similar. The cytoplasm forms a narrow band around a large nucleus and contains no granules. The nucleus contains from one to three nucleoli. The lymphoblasts are about the same size as the myeloblasts, making it difficult to differentiate between them.

Monoblasts are the parent cells of monocytes and are never found in the normal blood. Microscopically they cannot, as a rule, be distinguished from other cells.

RETICULOCYTE COUNTS

Materials:

1. Equipment for finger puncture.
2. Clean cover slips and slides.
3. 1% solution of brilliant cresyl blue in normal saline. FILTER BEFORE USE.

Procedure:

1. Into a clean centrifuge tube place equal quantities of fresh blood and the dye solution and mix well. Two or three drops are usually sufficient.
2. Let stand 10 minutes.
3. Make smears so the red cells will be almost touching each other, but not piled up.
4. Examine under oil immersion lens; or better, do a Wright's stain and then examine.
5. Count number of Reticulocytes per 1000 red cells. Normal counts will be .5% to 1.5%.

NOTE: It often assists in counting reticulocytes to fit a piece of paper covering the lower lens of the eye piece. In this cover cut a hole about $\frac{1}{4}$ inch square. This greatly reduces the visible area and is

more resting to the eyes.

PLATELET COUNT

Materials:

1. Finger puncture equipment.
2. Red blood cell pipette
3. Haemacytometer
4. Diluting fluid (Rees and Ecker's).

Sodium Citrate (3.8% acqueous solution)	100.0 cc.
Formalin	0.2 cc.
Brilliant cresyl blue	0.1 gm.

Procedure:

1. Rapid work is necessary to prevent clumping of platelets.
2. Draw blood to the 0.5 mark and Rees and Ecker's fluid to the 101 mark.
3. Shake immediately and thoroughly.
4. Fill the counting chamber as in making an erythrocyte count.
5. Allow to stand for 10 minutes so the platelets will settle out.
6. Examine 400 small squares and multiply by 2000 to obtain the number of platelets per cubic millimeter of blood. Use 10X ocular and 4 mm. objective.

NOTE: Platelets are irregularly small, lightly staining, highly refractile bodies. Normal counts are around 350,000 per c mm.

SEDIMENTATION RATE:

There are so many methods now in use for performing this test that results have not been comparable. Only two methods will be given here with the normals for these methods only.

Materials:

1. Venipuncture equipment.

2. Cutler tube. This is a tube of 1 cc. capacity and marked into fifty 1 mm. divisions with 0 at the top.

3. 2 cc. sterile syringe.

4. 3% Sodium Citrate Solution (sterile).

Procedure:

1. Draw 0.1 cc. of 3% Sodium Citrate into the 2 cc. syringe.

2. Draw into the same syringe 0.9 cc. of blood from the vein.

3. Mix and pour into the upright tube.

4. Read the height of the blood cell column every 5 minutes for 1 hour, plot results as a graph.

The normal for men is under 8 mm. and for women under 10 mm. in one hour, with a horizontal line.

(1) A diagonal line with a fall greater than normal, indicates a mild condition.

(2) Diagonal curve with the fall continued in the last half-hour indicates an active condition.

(3) Vertical curve with the entire fall in the first half-hour indicates a more severe condition.

In an effort to use materials normally on hand in the laboratory, the modified Cutler method is suggested. Select test tubes from 75 x 10 mm. Stock of such caliber that 2 cc. gives a column 50 mm. high, etch the tube at this point. Put exactly 0.2 cc. of 3% Sodium citrate in the tube. Fill to the 500 mark with blood. Mix by inverting, avoid air bubbles. Set tube in the vertical position. Measure with a millimeter ruler and record at 10, 20, 30minutes and 1 hour. Chart on a graph.

Normal for men, 2-8 mm. in 1 hour; for women, 2-10mm. The Sedimentation Method of Wintrobe and Landsburg has been found to be very good in that from it the cell volume may be determined also.

This requires a Wintrobe and Landsburg tube.

Procedure:

1. Fill the tube to the 10. mark with well mixed oxalated blood.
2. Put the tube in exactly a verticle position. A cork with a hole in it is excellent to put the tube in. Tube must not be moved or jarred during period of observation.
3. Read at the end of 1 hour. It can be read at 15 minute intervals. Normally the rate for males is 2 to 9 mm and for females 2 to 22 mm.

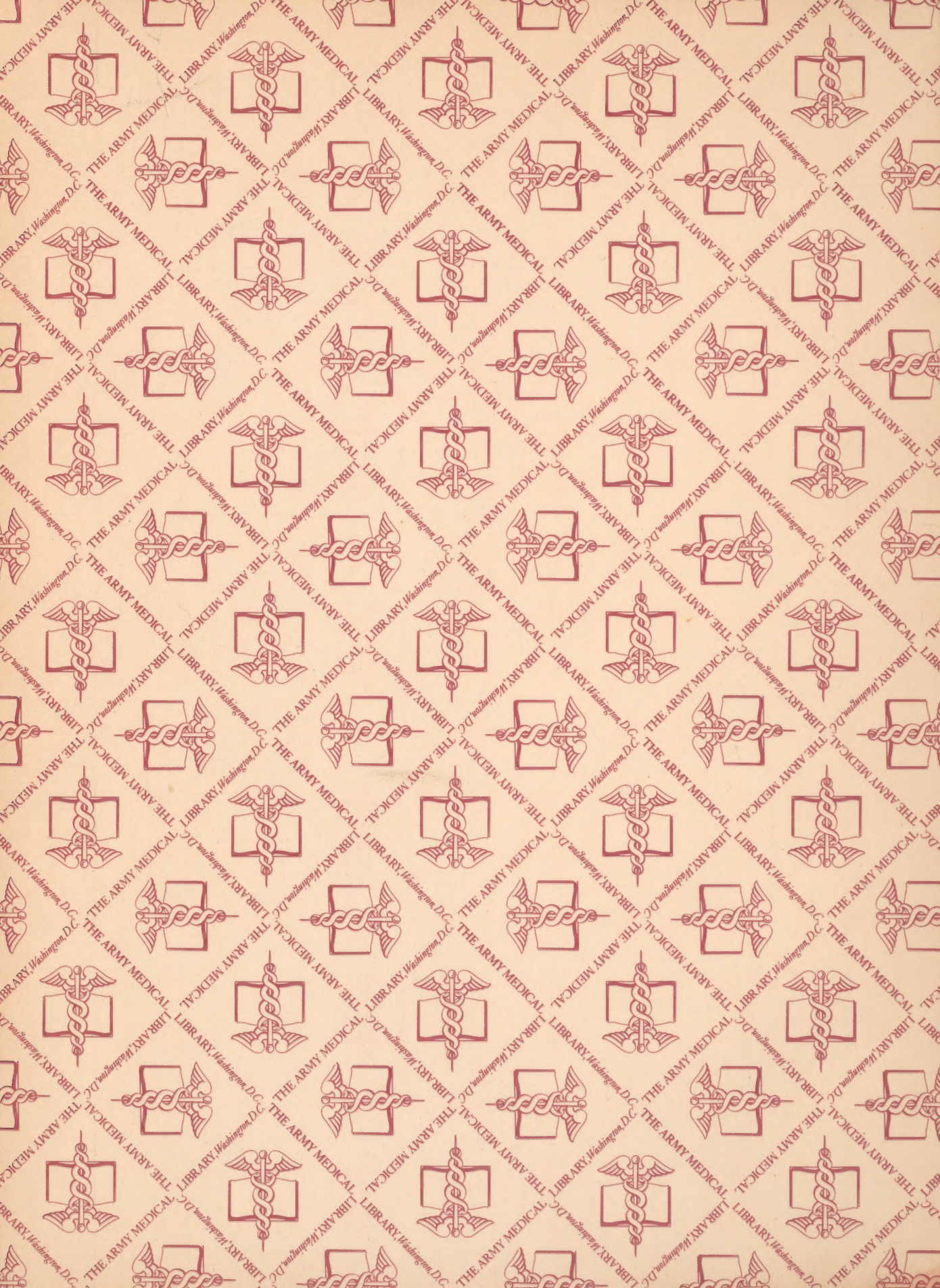
Volume of the Red Blood Cells:

Equipment:

1. Hematocrit tube of Wintrobe and Landsburg.
2. Venipuncture equipment
3. Tube with Oxalate. USE 2 mg. OXALATE PER cc BLOOD USED.

Procedure:

1. After the final reading for sedimentation rate centrifuge at 2500 RPM for 30 minutes.
2. Stop centrifuge and take reading.
3. Centrifuge for another 15 minutes.
4. If the reading is lower than at the end of 30 minutes centrifugalize again. If the reading remained constant, READ THE CELL VOLUME DIRECTLY IN PERCENT.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